




RESEARCH NOTE



Response of Antarctic sea-ice algae to an experimental decrease in pH: a preliminary analysis from chlorophyll fluorescence imaging of melting ice

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ABSTRACT

Microorganisms confined to annual sea ice in the Southern Ocean are exposed to highly variable oxygen and carbonate chemistry dynamics because of the seasonal increase in biomass and limited exchange with the underlying water column. For sea-ice algae, physiological stress is likely to be exacerbated when the ice melts; however, variation in carbonate speciation has rarely been monitored during this important state-transition. Using pulse amplitude modulated fluorometry (Imaging-PAM, Walz), we documented in situ changes in the maximum quantum yield of photosystem II (F_v/F_m) of sea-ice algae melting out into seawater with initial pH values ranging from 7.66 to 6.39. Although the process of ice-melt elevated seawater pH by 0.2–0.55 units, we observed a decrease in F_v/F_m between 0.02 and 0.06 for each unit drop in pH during real-time fluorescence imaging. These results are considered preliminary but provide context for including carbonate chemistry monitoring in the design of future sea ice state-transition experiments. Imaging-PAM is a reliable technology for determining F_v/F_m , but is of limited use for obtaining additional photosynthetic parameters when imaging melting ice.

KEYWORDS

Antarctica; imaging-PAM; photophysiology; carbonate chemistry

ABBREVIATIONS

I-PAM: imaging pulse amplitude modulated fluorometry; PAR: photosynthetically active radiation; UV-B: ultraviolet radiation

Introduction

High-latitude waters of the Southern Ocean will be among the first places on Earth to experience the effects of anthropogenic-induced climate change, including ocean warming and acidification (Fabry et al. 2008; Doney 2010). In the McMurdo Sound region of Antarctica, the mean seawater pH is currently 8.05, with a seasonal range of 0.42 units. By 2100 pH is predicted to drop below 7.9 and exhibit greater annual variability (Kapsenberg et al. 2015). The associated change in seawater carbonate speciation (increased CO_2 , HCO_3^- , H^+ ; decreased CO_3^{2-}) and its effect on polar ocean primary production are not fully resolved. Shelf waters surrounding Antarctica are effective sinks for anthropogenic carbon emissions, and increased oceanic CO_2 availability could facilitate faster rates of photosynthesis and growth in marine photoautotrophs (Riebesell et al. 1993). However, dissolved inorganic carbon is rarely in short supply in planktonic ecosystems and many taxa, including diatoms, possess carbon-concentrating-mechanisms that are highly efficient at elevating CO_2 at the site of RuBisCO (Reinfelder 2011; Kranz et al. 2015). To date, incubation experiments with Southern Ocean phytoplankton have demonstrated a variable response to elevated CO_2 (Müller et al. 2015).

Some phytoplankton down-regulate carbon-concentrating mechanisms, a response that potentially liberates energy that can be reallocated to other functions (Raven et al. 2011), but this does not always equate to increased growth rates and primary production (Torstensson et al. 2012; Trimborn et al. 2013; McMinn et al. 2014; Young et al. 2015).

For microbes confined to annual sea ice in the Southern Ocean the situation is significantly different. Because of the seasonal increase in biomass and limited exchange with the underlying water column, there are a number of dramatic chemical changes that take place within the ice matrix, specifically variable CO_2 , O_2 and pH dynamics (Thomas & Dieckmann 2002; Thomas & Papadimitriou 2003). Efficient physiological acclimation that sustains photosynthesis within the ice is therefore critically important (Arrigo & Thomas 2004; McMinn et al. 2005), but the influence of pH on algal physiology is not clearly understood. Experimental work with cells growing near the upper surface of the ice demonstrates that increased CO_2 enhances growth by approximately 20%, provided that cells are incubated at a constant pH of ca. 8.0 (McMinn et al. 2014). If microbes are exposed to decreasing pH (< ca. 7.2), then the increase in H^+ ions has been shown to reduce growth rates by almost 50% (McMinn et al. 2014).

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A general understanding of how sea ice impacts the carbon cycle and ocean–atmosphere CO_2 exchange in polar regions is now emerging (see Rysgaard et al. 2011; Fransson et al. 2015), but the associated changes in carbonate chemistry due to melting ice remain poorly characterized (Bates et al. 2014). The bottom-ice algae that dominate the biological assemblages in Antarctic fast-ice undergo considerable physiological stress during the melt process. This ecological state-transition has been well studied with respect to UV-B and PAR (e.g., Palmisano & Sullivan 1983; Mock & Kroon 2002; McMinn et al. 2010; Ryan et al. 2012), temperature (e.g., Arrigo & Thomas 2004; Mock & Thomas 2005; Ralph et al. 2005) and salinity (e.g., Arrigo & Sullivan 1992; Ralph et al. 2007; Ryan et al. 2011; Ugalde et al. 2014), but not to associated changes in pH. The evolution of distinct pH environments within sea ice has only recently been described by utilizing an experimental outdoor facility (Hare et al. 2013). Analysis of vertical pH profiles revealed a consistent C-shaped pattern with highest pH values (>9) in both top and bottom sections of the ice and lowest pH (ca. 7) in interior ice sections, albeit in the absence of biological activity. In spring and summer, microbial photosynthetic activity depletes dissolved CO_2 and pH rises. However, it is not known how variation in pH influences algal photophysiology with respect to freeze–thaw dynamics.

A particularly useful tool for correlating algal photophysiology with changing environmental conditions has been pulse amplitude modulated fluorometry. This technique is based on measuring variable chlorophyll *a* fluorescence, which provides a unique indicator of intracellular activity in photoautotrophs (Schreiber 2004; Ralph & Gademan 2005; Baker 2008). McMinn et al. (2003) pioneered the use of pulse amplitude modulated fluorometry in Antarctica, and various configurations of the

technology have now been employed for the *in vitro* analysis of ice shavings, extracted brines and melted ice cores (e.g., Ryan et al. 2004; Ralph et al. 2007; Kennedy et al. 2012; Martin et al. 2012). I-PAM (Walz, Germany) provides unprecedented two-dimensional *in situ* imagery which allows physiological stress to be measured with significantly less disruption of the physical habitat (Ryan et al. 2011; Hawes et al. 2012; Lund-Hansen et al. 2014). This technology has only been employed once in Antarctica and with mixed success (Ryan et al. 2011), but the capacity to generate *in situ* data warrants further investigation. In this preliminary study, our objectives were to expose sea-ice algae to changes in pH to determine whether short-term (8-h) exposure to reduced pH influences algal photophysiology (Experiment 1) and to quantify changes in pH that occur during the melt process (Experiment 2). We also determine whether imaging fluorescence is a viable technology to resolve changes in algal photophysiology during state-transition experiments.

Materials and methods

Study area

Bottom-ice algae were collected from 1.8 m thick annual fast-ice at Cape Evans, McMurdo Sound, Antarctica ($77^{\circ}38'S$, $166^{\circ}24'E$), between 27 November and 1 December 2013 (Fig. 1). Limited incubation space in the field laboratory required that the experiment be carried out over four days; one day per pH treatment and one day for the control. The order of the treatments and control was selected at random to minimize any effect of *in situ* change in the microbial community. Two ice cores were required each day and these were obtained by drilling to within 300 mm of the ice/water interface using a powered ice auger (Jiffy), and then using a

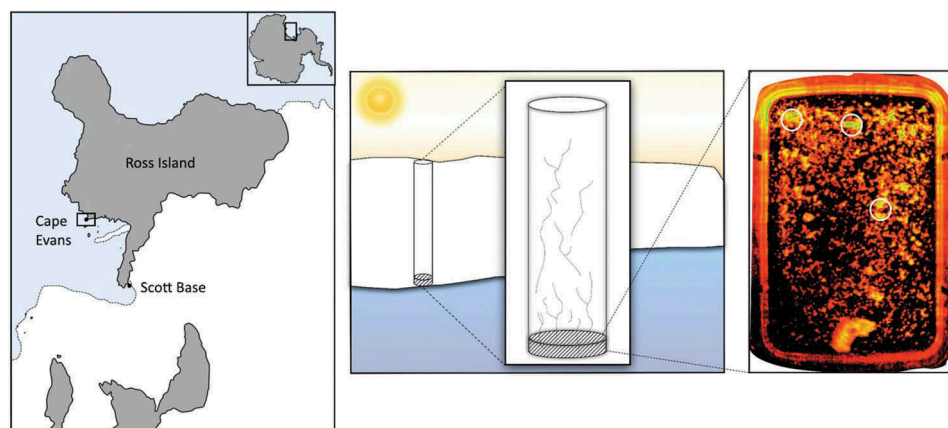


Figure 1. Location and schematic representation of sampling site at Cape Evans, McMurdo Sound, Antarctica. The image on the right shows a typical ice sample melting into manipulated seawater. The ice has been imaged using minimal fluorescence, F_o , with an I-PAM. The circles represent regions of relatively high algal biomass.

Mark V corer (Kovacs) to extract the last section of ice from the bottom of each hole. Once clear of the ice, each core was immediately transferred to a black polythene bag to protect the algae from light shock and transported a short distance to the field laboratory inside a cool box.

Experimental set-up

Each day, the two extracted ice cores were cut, under minimal light, into three pieces 40 mm × 40 mm × 20 mm thick to include bottom-ice algae. The six blocks of ice were then dark-adapted for 30 min in a cool box maintained at about 0°C before being placed into individual airtight plastic containers with clear plastic covers containing 112 ml of seawater (filtered to 0.7 µm, −1.8°C). The seawater had previously been collected from a local dive hole. Seawater pH was reduced by adding HCl which changed the total alkalinity (A_T) while keeping the dissolved inorganic carbon (C_T) concentration relatively constant. Untreated seawater was used as a control treatment (pH 7.66, pCO₂ 1651 µatm). The three pH treatments ranged from 7.32 to 6.39 (free scale). During the daily incubation, the six plastic containers containing ice core samples were placed in a temperature controlled water bath at 0.5°C ± 0.2°C for 480 min. An artificial light source comprising a 3 × 4 array of LED lamps suspended 800 mm above the water bath was used to provide an incubation irradiance of 40 µmol photons m^{−2} s^{−1}. Three of the plastic containers were periodically opened in order to assess algal photophysiology (Experiment 1). Constant agitation of the seawater inside the containers (generated by circulation of the liquid in the water bath) ensured that the ice was bathed in seawater and prevented stratification as the ice melted. The other three containers, also exposed to light, remained closed for the duration of the experiment to examine the change in carbonate chemistry due to the melt process and biological activity (Experiment 2).

Experiment 1: photophysiology

Variable chlorophyll fluorescence was measured using the Maxi version of the I-PAM instrument (Walz). The I-PAM Maxi can resolve the distribution of chlorophyll *a* and activity of photosystem II in two dimensions over an area of 130 mm × 100 mm and the user has the option of employing pulse-modulated excitation for the generation of rapid light curves, actinic light and saturation pulses. The blue excitation light supplied by the I-PAM is centred on 450 nm, which is a wavelength that is characteristic of the blue-green spectra at the bottom of the ice (Lund-Hansen et al. 2014). The saturation pulse method was used to determine minimum (F_o) and maximum (F_m) fluorescent yields (F_v/F_m) of the dark-adapted ice

samples. The I-PAM operational software includes an area-of-interest function that was used to select three specific locations on each block of ice to obtain F_v/F_m measurements. Selecting locations on each block of ice was a semi-random process; data were only acquired from areas-of-interest where F_o was >0.15. F_o is a measure of minimum fluorescence intensity of algae in the dark-adapted state where intensity is proportional to biomass. Fluorescent measurements of the blocks of ice were made by first removing the lid and then carefully aligning each plastic container on the stage of the I-PAM by using the live near-infrared imaging feature. After obtaining fluorescence measurements the lid was placed back on the container and immediately returned to the water bath. This process was performed in the dark in a field laboratory maintained at ca. 4°C and took no more than 30 s per sample. An initial assessment of F_v/F_m was made before the ice was added to the containers and then at 30-min intervals up to 120 min. Thereafter, fluorescence imaging was carried out at 240, 360 and 480 min. Samples were dark-adapted in the water bath for 10 min prior to fluorescence analysis (10–15 min is typical for dark-adapting sea ice algae (Ralph et al. 2007; McMinn et al. 2010)) by turning off the overhead light array. The generation of rapid light curves was attempted, but like Ryan et al. (2011), we could not achieve the required saturation to derive the necessary parameters.

Experiment 2: carbonate chemistry

Samples for C_T and A_T were taken from replicates of the melted ice at the end of each experimental run. C_T samples were filtered through 0.2 µm sterile filters into 12.5 ml Exetainers (Labco), poisoned with HgCl₂ and sealed air tight. A_T samples were filtered through GF/F filters, poisoned with HgCl₂ and stored in 250 ml polythene flasks. The programme CO₂SYS version 1.05 (Lewis & Wallace 1998) was used to calculate the carbonate system using the dissociation constants for carbonic acid (Roy et al. 1993). C_T and A_T samples were analysed as the mean of triplicate measurements with the infrared detection method using a dissolved inorganic carbon analyser (Apollo SciTech, model AS-C3) and the potentiometric titration method for A_T . Data were corrected to Certified Reference Materials (Scripps Institution of Oceanography, USA). Consecutive measurements of the Dickson standard resulted in an average precision of >98% for both C_T and A_T .

Ice algal species composition

A 10 ml sample of three randomly selected core sections was fixed in 0.5% glutaraldehyde. A 0.5 ml

aliquot from each sample was prepared using a special settling chamber and cells were examined using a Zeiss compound microscope with standard optics. Microalgae were counted from 10 randomly selected fields of view.

Data analysis

To examine potential changes in maximum quantum yield (F_v/F_m) relative to pH and incubation period, a linear third-order polynomial model was fitted to the data using the statistical package RStudio (version 0.98.939; RStudio Team).

Results and discussion

This is the first study to employ chlorophyll imaging fluorometry to examine the effect of decreased pH on algal photophysiology. Experiments were conducted in a remote Antarctic field camp with limited facilities to replicate a precisely controlled laboratory environment. A broad initial pH regime was chosen (6.39–7.66) to allow for shifts in carbonate chemistry induced by outgassing and experimental handling procedures, specifically the removal of plastic lids to perform I-PAM measurements, which exposed ice samples to the atmosphere (3.5 min during the 8-h incubation, Experiment 1).

The sea ice at Cape Evans in McMurdo Sound was 1.8 m thick in November–December 2013, with no snow cover. The bottom-ice community was dominated by the pennate diatom *Nitzschia stellata*, which accounted for >90% of the observed cells. Other taxa present within the ice included *Fragilariopsis* spp., *Gyrosigma* sp., *Navicula directa* and *N. glaciei*.

Photophysiology

The ratio F_v/F_m derived using the saturation pulse method is a sensitive indicator of photosynthetic potential in photoautotrophs. Prior to incubation, F_v/F_m of the sea-ice algae ranged from 0.37 to 0.52, which is indicative of a relatively healthy community that was well-acclimated to the light climate within the ice matrix (Table 1). At the incubation temperature of 0.5°C, it took approximately two hours for the ice to completely melt, during which time the ice was imaged four times. The post-melt F_v/F_m values obtained at 240, 360 and 480 min were derived from algae that were suspended in seawater (Fig. 1). The maximum quantum yield of photosystem II declined in all treatments and the control during the period of ice-melt (Table 1). This response is indicative of the physiological stress associated with both the transition from semi-solid ice to liquid water and exposure to light. A similar response was documented by Ryan et al. (2011) when sea-ice algae were

Table 1. Response of sea-ice algae exposed to variable pH during ice-melt. Maximum quantum yield (F_v/F_m) was determined using imaging fluorescence (I-PAM). Each value is the mean with standard error in parentheses, $n = 3$.

Time (min)	Initial pH _{free}			
	6.39	6.84	7.32	Control (7.66)
0	0.52 (0.01)	0.49 (0.01)	0.49 (0.02)	0.37 (0.02)
30	0.50 (0.02)	0.45 (0.02)	0.46 (0.03)	0.36 (0.02)
60	0.50 (0.02)	0.47 (0.01)	0.44 (0.02)	0.37 (0.02)
90	0.51 (0.03)	0.47 (0.01)	0.43 (0.02)	0.37 (0.02)
120	0.50 (0.02)	0.42 (0.01)	0.41 (0.01)	0.35 (0.04)
240	0.46 (0.02)	0.45 (0.01)	0.45 (0.01)	0.35 (0.03)
360	0.45 (0.01)	0.43 (0.01)	0.41 (0.02)	0.35 (0.04)
480	0.44 (0.02)	0.42 (0.02)	0.43 (0.02)	0.34 (0.02)

first examined using imaging fluorescence. These authors also identified salinity to be a significant co-stressor, particularly at concentrations <24. To prevent saline shock in the current study, we used smaller blocks of ice. As a result, the salinity only decreased from 34 to ca. 28 by the end of the experiment (Table 2) and any effect on F_v/F_m is considered to be minimal. To determine whether pH also influenced the photosynthetic yield measurements, we fitted a linear third-order polynomial model to the fluorescence data. This model accounted for the variation in initial F_v/F_m that was evident in the pH 7.66 treatment (Table 2). By retaining only the first order term in the model, the analysis revealed that for each unit drop in pH, the change in yield of photosystem II decreased over time by between 0.02 and 0.06 ($t(10) = 4.06$, $r^2 = 0.623$, $p < 0.01$; Fig. 2).

Experimental manipulation of pH has two main effects on marine photoautotrophs, specifically acidification (increased H^+) and carbonation (elevated CO_2). Relative to ambient oceanic conditions, the former generally has a negative effect on algal physiology, the latter a positive effect. This results in an optimum curve with respect to the physiological response in marine microbes (see Bach et al. 2015). McMinin et al. (2014) were the first to examine how algae released from the ice matrix respond to manipulated carbonate chemistry, and while a decline in F_v/F_m was observed in brine algae exposed to a pH regime that varied from 8.66 to 7.19, the trend was not significant. More recently, Coad et al. (2016) melted algae from the snow/ice interface region of annual pack-ice into a pH regime that varied from 7.82 to 7.0, but only modest impacts on photophysiology were documented after seven days. Because of this non-response in photosynthetic parameters, McMinin et al. (2017) have questioned the use of pulse amplitude modulated fluorometry to measure stress response in pH/ CO_2 experiments. In this study, we adopted a more acidic regime and did observe a significant response to reduced pH. However, the relationship between H^+ concentration and F_v/F_m has yet to be substantiated. Previous studies that have included growth rates (e.g., McMinin et al. 2014; McMinin et al. 2017) have shown

Table 2. Carbonate chemistry of CO₂ manipulation experiment utilizing bottom-ice algae at Cape Evan, 2013. The end of experiment data (8-h incubation) illustrates the changes in carbonate chemistry associated with ice-melt. The unit for C_T, A_T and CO₂ is $\mu\text{mol kg}^{-1}$. The unit for pCO₂ is μatm .

Initial seawater chemistry									
pH _{free}	Salinity			C _T	SD	A _T	SD	pCO ₂	CO ₂
Control 7.66	34.67			2303	9	2396	40	1651	44
Start of experiment		End of experiment							
pH _{free}	Salinity	pH _{free}	Salinity	C _T	SD	A _T	SD	pCO ₂	CO ₂
Control 7.66	34.67	Control 8.15	28.07	2175	220	2493	50	593	16
7.32	34.73	7.52	27.33	2230	260	2282	114	4335	120
6.84	34.70	7.14	30.00	1983	175	1860	19	6547	174
6.39	34.80	6.94	28.67	1879	101	1879	180	10,177	273

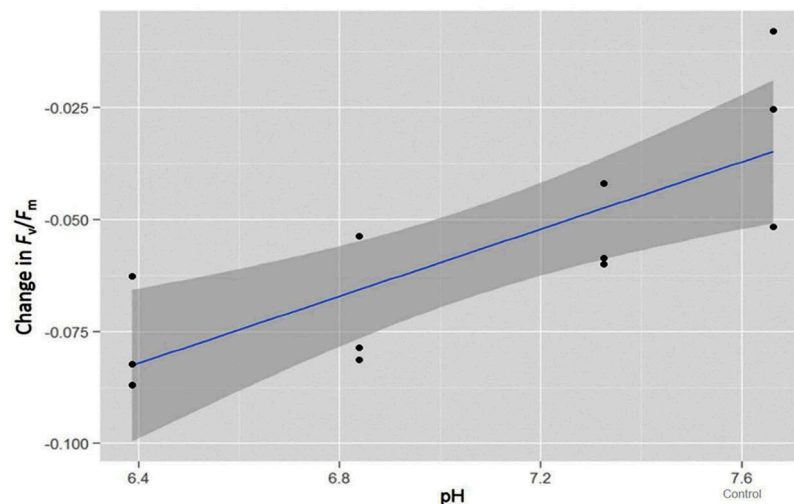


Figure 2. Linear third-order polynomial model of maximum quantum yield (F_v/F_m) for each pH treatment and the control contrasting the difference between start and end-point measurements. The error variance is represented by the dark grey band.

that a decline in growth is attributed to high H⁺ concentration rather than high CO₂. This suggests that H⁺ concentration can influence intracellular pH and/or changes in membrane potential, energy partitioning and enzyme activity (Beardall & Raven 2004; Suffrian et al. 2011), and this has a subsequent effect on photosynthetic performance. Although the decline in photosynthetic potential observed during ice-melt was most pronounced in cells exposed to the lowest pH (6.39), F_v/F_m was consistently higher compared to the less acidic treatments and the control (Table 2). This highlights the fact that a significant increase in H⁺ ions has a minimal short-term effect on F_v/F_m , as assessed using an I-PAM. The relative change in F_v/F_m described by the model must be considered a subtle trend, but it is potentially indicative of a more significant stress response had the experiment been conducted over a longer time period. Importantly, we did not observe a threshold at which point algal photophysiology is compromised to the extent observed when other stressors such as light (Ralph et al. 2007; Ryan et al. 2009; Ryan et al. 2011), temperature (Ralph et al. 2005; Rajanahally et al. 2014) or salinity (Ryan et al. 2004; Ralph et al. 2007; Ryan et al. 2011) are manipulated.

Carbonate chemistry

The aim of Experiment 2 was to provide a rudimentary insight into the changes in pH that occur as the sea ice melts. The post-incubation carbon chemistry data (Table 2) was obtained from plastic containers that were not opened during the incubation period and for that reason the data are not directly comparable with the photophysiology experiment. By the end of the incubation period, ice-melt and the biological activity of liberated microbes had increased the seawater pH in all treatments and the control, from 0.2 to 0.55 units. This provides a very basic illustration of the dynamic that occurs at the receding ice edge which has recently been described at an ecologically relevant scale in the Arctic Ocean as part of the NASA ICESCAPE project (Bates et al. 2014). The carbonate chemistry of below-ice interface melt waters at 19 ice stations was variable and difficult to predict, but generally exhibited higher pH and lower pCO₂ compared to the co-located mixed layer beneath. Although we collected ice cores from a discrete area of sea ice, there was significant variation in post-melt measurements of C_T and A_T, presumably due to variability in biomass and the ratio of ice to

brine volume in each block of ice. However, algal cellular physiology is not directly affected by variation in C_T and A_T ; of significance is the change in the total concentration of ions (CO_2 , HCO_3^- , CO_3^{2-} and H^+) that are made available.

In situ fluorescence imaging

The practice of actively releasing cells from the ice matrix is likely to modify or damage cellular physiology and ice-associated microbes should ideally be studied in situ. While this has been successfully achieved using oxygen electrodes (e.g., Kühl et al. 2001; Trenerry et al. 2002; McMinn et al. 2012) and habitat modification experiments (Martin et al. 2011), options for physiology experiments are limited. In situ fluorescence imaging is of significant interest because algal photophysiology can be assessed at a spatially relevant scale, specifically the assessment of cells inside the microscopic brine channels that characterise the ice matrix. Although our measurements are only indicative of ablation at the receding ice edge in the austral summer, real-time measurements are considered important because this state-transition, and exposure to physico-chemical stress, occurs relatively quickly. Like Ryan et al. (2011), we could not use the I-PAM to generate rapid light curves and obtain the additional photosynthetic parameters $rETR_{\text{max}}$, α , and E_k . The maximum rate of electron flow from photosystem II to photosystem I (ETR_{max}) is of particular interest because it describes photosynthetic capacity. The initial ice dimensions used by Ryan et al. (2011) were 70 mm × 60 mm × 15 mm and we reduced this to 40 mm × 40 mm × 20 mm (i.e., a 50% reduction in volume). However, the relative abundance of algae within the ice was still too high. The rapid light curves do not reach an asymptote because multiple layers of densely packed cells confound the required response to the increasing irradiance emitted by the fluorometer. To an extent, this problem has now been addressed by Hawes et al. (2012), who successfully generated rapid light curves RLCs during fluorescence imaging of newly forming Arctic sea ice with a relatively low biomass. These authors used the I-PAM Mini (Walz), which has a reduced imaging area of 30 × 23 mm, and integrated the fluorescence data obtained from a series of ice core sections that were 2 mm thick. Working with such thin sections is not a practical solution in state-transition experiments because of rapid ice-melt. For this reason we suggest the I-PAM Maxi is only relevant for collecting in situ measurements of maximum quantum yield (F_v/F_m).

Conclusion

This is the first study to document statistically significant photophysiological stress in sea-ice algae due to decreased pH. Future experiments will require greater experimental precision, but the data provide context for assessing the relative importance of carbonate chemistry to sea-ice ecophysiology. Importantly, the physiological stress associated with rapid exposure to high light and low salinity during ice-melt is significant and the compound stress associated with the projected decline in pH in Antarctic coastal waters has the potential to shift environmental conditions beyond physiologically tolerable levels (McMinn et al. 2014). A mechanistic understanding of how ice-associated microbes adapt to freeze-thaw dynamics requires a clearer insight into how pH influences algal physiology. For this reason, we suggest that carbonate chemistry monitoring should be included in the design of future multi-stressor state-transition experiments.

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Disclosure statement

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